Quantitative Histological Analysis of the Human Sinoatrial Node During Growth and Aging

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Background. Fibrosis or fatty infiltration of the human sinoatrial (SA) node is generally believed to represent replacement of the SA nodal cells by connective tissue. Quantitative analysis, however, has not been performed precisely to validate the interpretation of such histological changes.

Methods and Results. The actual volume of the SA node and its components were calculated according to the sum of the pixel number representing the colors of SA nodal cells and connective tissue in serial sections using a digital color image analyzer. Average volume occupied by the total SA nodal cells in adolescents and adults (n=7) was 3.55±0.45 mm³, which was 2.4 times greater than that in infants (n=6). The rate of increase was smaller than that of the total SA node (4.2 times, 16.68±2.56 mm³ in adolescents and adults). The considerable discrepancy in the growth ratio between the SA nodal cells and the total SA node resulted from an increase in the volume of connective tissue (7.4 times). In the elderly (n=9), the volume of total SA node and SA nodal cells actually decreased (13.10±1.85 mm³ and 2.18±0.44 mm³), whereas that of fibrous connective tissue remained unchanged. Constant DNA ploidy patterns of SA nodal cells determined by cytofluorometry indicated that SA nodal cells never synthesize DNA during growth.

Conclusions. Until adulthood, the actual volume of SA nodal cells does not decrease, although the increase in volume ratio of the interstitial tissue to the total SA node has merely given a false impression of involution of SA nodal cells. Atrophy of SA nodal cells, however, occurs during aging together with reduction of the SA node and/or infiltration of fatty tissue. (Circulation 1992;85:2176-2184)

Key Words • color image analyzer • cytofluorometry • DNA ploidy pattern

The histology of the human sinoatrial (SA) node is characterized by a conspicuous mixture of specialized myocardium (SA nodal cell) and the abundant fibrous connective tissue.1-6 A collagen network is observed even in children, but it becomes more apparent with age.2,3 It has been generally believed that the increase in quantity of the connective tissue is at the expense of SA nodal cells.4-10 Actually, SA nodal dysfunction increases in the elderly, and its incidence is supposedly related to the degree of increasing amount of connective tissues.9-11

Many reports concerning quantitative histological analysis of the SA node were based on the point-counting method,5-10 which can demonstrate a relative percentage of SA nodal cells and connective tissues in a somewhat limited area of the SA node. If the volume of SA node remained constant during growth and aging, the decrease in the percentage of SA nodal cells should indicate a loss of the SA nodal cells. Although the length, width, and thickness of the SA node are by no means constant during growth,12,13 the actual volume of the SA node has never been measured.

In the present investigation, we studied whether the actual volume of the SA nodal cells decreases during growth and aging. Using serial sections and a digital color image analyzer, the actual volumes of the total SA node, SA nodal cells, and connective tissue were calculated. The DNA contents of the SA nodal cells were then determined by Feulgen cytofluorometry. The actual volume of SA nodal cells increased during growth, although its volume percentage in the total SA node decreased because of the prominent volume increase of fibrous connective tissue. On the contrary, the total volume and volume percentage of SA nodal cells virtually decreased during aging, and the volume of fibrous connective tissue remained unchanged. We conclude that the increase in volume ratio of the fibrous connective tissue during growth has merely given a false impression of involution of SA nodal cell.

Methods

Color Image Analysis

The subjects in this study consisted of 26 autopsy hearts taken from patients with no documented history of arrhythmias (Table 1). They included 17 male subjects and nine female subjects ranging in age from 3 months to 89 years. These patients were divided into

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four groups for analysis: infants (age <1 year, cases 1–3, n=3), children (age, 1–15 years; cases 4–10, n=7), adolescents and adults (age, 16–50 years; cases 11–17, n=7), and elderly patients (age >50 years, cases 18–26, n=9).

The sinoatrial junction areas were removed from the autopsy hearts according to the method reported by Lev. They were fixed in 10% formalin, cut into three to five blocks, and embedded in paraffin. To correct the thickness of each block after serial sectioning, a Teflon tube 3 mm in length was also embedded. Serial sections with a thickness of 4 μm were prepared parallel to the long axis of the SA node. Every 10th section was cut into a thickness of 200 μm. The thin sections were stained with Masson’s trichrome method to differentiate the SA nodal cells from collagen, elastic fibers, and other connective components.

The system for color image analysis consists of a charge-coupled device video camera (Sony DXC325), a color image analyzer (Nexus 6000), and a host computer (Hewlett-Packard HP9000). A color image was taken through a microscope (Olympus AH2, objective lens: SPlan-Apo ×10) with the video camera and digitized by an eight-bit analog/digital converter. The images were then stored in the frame memory boards (512×480×8 bits) that were configured to correspond to the red, green, and blue primary images of a normal color picture (Figure 1A). They were displayed on the cathode ray tube (CRT) at the actual magnification of ×193.

The region of the SA node to be examined was extracted on each image with a digitizer (Figure 1B). There were narrow transitional zones between the compact SA node and the surrounding working myocardial cells of the right atrium. The transitional zone was excluded in order to restrict the area of interest to the compact portion composed of typical SA nodal cells. The area of the SA node was calculated from a total number of pixels within the remaining images. After removing the region of the SA nodal artery, a color range specialized to the SA nodal cell was picked up with the digitizer. All pixels within this color range were then converted to red (Figure 1C). The area of the SA nodal cells was determined by counting the number of red pixels. The area of the fibrous connective tissue was calculated after converting their color range to blue (Figure 1D).

The volumes of the total SA node, the SA nodal cells, and the connective tissue were calculated by adding the pixel numbers from every 10th section covering the entire SA node.

**Cytofluorometry**

To examine the nuclear DNA ploidy pattern of the SA nodal cell, DNA cytofluorometry was performed on isolated SA nodal cells and working myocardial cells taken from the right atrium of three patients without arrhythmia. These patients included a 3-month-old infant, a 7-year-old child, and a 39-year-old adult. Speci-
mens were fixed with Carnoy's solution to avoid non-specific fluorescence. The region of the SA node was initially identified on a thin section and removed from the adjacent thick sections (200 μm) under a stereomicroscope. SA nodal cells were isolated by enzymatic digestion with 0.05% of type IV collagenase (Sigma) for 2 hours at 37°C and 0.01% of proteinase K (Merck) for 1 hour, followed by homogenization with a Potter-type homogenizer (five to 10 strokes). Smears of the isolated cells were stained with azocarmine G and acriflavine–Feulgen reaction. Cytofluorometric DNA determinations were made on about 100 nuclei in each smear with an Olympus MMSP-RF-KF cytofluorometer system. The scale of fluorescent intensity of diploid (2C) nuclei was adjusted to correspond to that of the nuclei of connective cells, such as fibrocytes. The nuclear DNA contents of the surrounding right atrial myocardium were also measured in the same manner.

Results

Identification of the SA Node and its Margins
Most of the SA nodes were located at the upper part of the sulcus terminalis. The SA nodal artery enabled us to identify the location of the SA node. Usually, the SA node was easily distinguishable from the surrounding structures because of its pale appearance. The node showed a plexiform network consisting of slender muscle fibers and fibrous connective tissues. The nodal cells have light-staining cytoplasm and uniform, smaller nuclei than those of the surrounding atrial working myocardial cells. The cells were embedded in a thick mass of collagen and elastic fibers that had

progressively increased with age. Mitosis was not observed at all in the SA node of any age group that was studied.

The margins of each SA node were fairly clear; nevertheless, narrow transitional zones were present (Figures 1 and 2). The SA node was located just beneath the epicardium. The upper portion of the SA node was bordered by loose connective tissue continuous to that of the superior vena cava. In the lateral side bordering the endocardium, there was a narrow transitional zone where thick working muscle cells and slender SA nodal cells, including pacemaker cells and transitional cells, were mixed. To compare the area and volume of each subject precisely, the present study intentionally excluded the transitional zones.

Representative images of the SA nodal cells and connective tissue taken from an infant (case 2), an adolescent (case 13), and an elderly patient (case 24) are serially shown in Figure 2, panels A, B, and C. Every SA node in infants, children, adolescents, and adults was spindle-shaped or arrowhead-like (Figures 2A and 2B). In the elderly, the peripheral areas of SA node were infiltrated with various degrees of fatty tissue. This change was most remarkable in the lateral side adjacent to the epicardium. The shape of elderly SA node, in contrast to those until adulthood, was irregular polygonal (Figure 2C).

Focal hemorrhage was recognized in some of the SA node as described in Hudson’s report. Amyloid infiltration was seen in some of the elderly SA node. This study excluded these cases from volume analysis because it was very difficult to determine the margin of the SA node and to pick up the specific color range of the SA nodal cells.

**Volume Analysis of the SA Node**

The measured maximal length, width, and thickness of the compact SA nodes were 3-5 mm, 2-4 mm, and 1.0 mm in infants and 8-10 mm, 4-5 mm, and 1.2-1.6 mm in adolescents and adults, respectively. At a magnification of ×193 on the CRT (objective lens ×10), the SA node extended over two or three images in infants and five or six images in adolescents and adults. The images had enough spatial resolution to distinguish the SA nodal cells from collagen fibers. Higher magnifications such as those taken by an objective lens ×20 or ×40 revealed no differences in volume (preliminary data), and moreover, made the analysis extremely laborious. In the present study, the total number of the analyzed sections and images that covered the entire SA node was 12-15 and 22-36 for each infant and 20-29 and 54-111 for each adolescent and adult, respectively.

The results are summarized in Figure 3. For the precise volume analysis, the SA nodal artery was excluded. The mean volume and standard deviation of the total SA node, the SA nodal cells, and the fibrous connective tissue in each group are summarized in Figures 4, 5A, and 6A, respectively. The volume ratios of SA nodal cells and fibrous connective tissue to the total SA node are also summarized in Figures 5B and 6B.

**Volume Analysis in Infants, Children, and Adolescents and Adults**

The volume of the total SA node increased with growth; the mean volume of the total SA node was 3.94±0.48 mm³ in infants, 8.59±2.01 mm³ in children, and 16.68±2.56 mm³ in adolescents and adults (Figure 4). The mean volume of the total SA node in adolescents and adults was 4.2 times \((p<0.001)\) greater than that in infants and 1.9 times \((p<0.01)\) greater than that in children (Figure 4), whereas the mean value of the heart weight in adolescents and adults (289.3±83.5 g) was 7.6 times \((p<0.001)\) greater than that in infants (38.3±6.2 g) and 2.2 times \((p<0.01)\) greater than that in children (133.6±48.9 g).

The volume of SA nodal cells also increased with growth: 1.50±0.16 mm³ in infants, 2.45±0.33 mm³ in children, and 3.55±0.45 mm³ in adolescents and adults (Figure 5A). The mean volume of SA nodal cells in adolescents and adults was 2.4 times greater than that in infants \((p<0.001)\) and 1.4 times greater than that in children \((p<0.001)\). The connective tissue increased more in volume (Figure 6A) and in ratio (Figure 6B); the volume in adolescents and adults \((7.20±1.28 mm³)\) was 7.4 times greater than that of infants \((0.97±0.25 mm³)\) and 2.6 times greater than that of children \((2.77±0.72 mm³)\).

In response to the increase in connective tissue, the volume ratio of SA nodal cells to the total SA node of adolescents and adults decreased (Figure 5B); the ratios in infants, children, and adolescents and adults were 39.0±7.3%, 29.7±5.8%, and 21.5±2.9%, respectively.

**Volume Analysis in the Elderly**

The actual volumes of total SA node and SA nodal cells in the elderly significantly decreased compared with those of adolescents and adults; the volumes were 13.10±1.85 mm³ \((p<0.01)\) and 2.17±0.44 mm³ \((p<0.01)\), respectively (Figures 4 and 5A). The volume ratio of the SA nodal cells also decreased (Figure 5B); 16.6±2.3%, \(p<0.01\). The total volume of fibrous connective tissue did not significantly change during aging (Figure 6A, 6.52±1.50 mm³); however, the volume ratio of fibrous connective tissue to the total SA node increased (Figure 6B; 49.3±5.8%, \(p<0.05\)).

During growth and aging, the volume change of extracellular space, small vessels, and intranodal small fat tissue was in proportion to that of the total SA node; the volume significantly increased during growth and decreased during aging \((p<0.05, \text{not shown})\).

The linear regression analysis between the volumes of each SA nodal component and the heart weight showed that the volume of total SA nodal cells and that of fibrous connective tissue during normal growth and aging were well correlated with the heart weight \((r=0.76 \text{ and } 0.74, \text{respectively; } p<0.001)\). However, the correlation between the volume of SA nodal cells and the heart weight was moderate \((r=0.42, p<0.05)\).

**DNA Determination of SA Nodal Cells**

Table 2 shows the nuclear DNA distribution in a 3-month-old infant, a 7-year-old child, and a 39-year-old adult. Compared with myocardial cells of the right atrium in the working heart, where polypliodization including octaploid (8C) nuclei occurred in the adult case, the SA nodal cells were composed of a large number of diploid and a small number of tetraploid cells. There were no intermediate cells between 2C,
4C, and 8C cells. Cytofluorometric nuclear DNA determination could be carried out in only three cases because the dense network of collagen and elastic fibers of the SA node prevented cell dispersion in most cases.

Discussion

Margins of the SA Node

The major problem in measuring the area and volume of the SA node is to determine the border of each SA node. Narrow transitional zones exist between the SA node and the surrounding tissue. The nodes are composed of mixtures of slender SA nodal cells and thick right atrial working myocardial cells. The margins between the compact SA node and the transitional zones were distinguishable. However, the margins between the transitional zones and the surrounding right atrial working myocardium were quite obscure. In the present study, the compact portion consisting of typical SA nodal cells was examined and the transitional zones were excluded in order to compare precisely the area and volume of each case.

The margins of the SA node were quite obscure in neonates. Neither size nor color of the neonatal SA nodal cells was distinguishable from the surrounding right atrial myocardium. Therefore, data for neonates

Figure 2. Facing page: Photomicrographs (left, Masson’s trichrome stain) and serial digitized images of sinoatrial (SA) nodal cells (right upper series) and connective tissue (right lower series) of every 1,500 μm (every 50th section). Right side of each image indicates epicardial side. Arrowheads indicate the border of SA node. Digitized images with asterisks correspond to photomicrographs on left in each case. Panel A: SA node of a 7-month-old infant (case 2). The sizes of SA nodal cells are similar to those of surrounding right atrial working heart muscle cells. There are a small number of collagen fibers in the node. Panel B: SA node of an 18-year-old adolescent (case 13). There is a difference in size between the SA nodal and right atrial muscle fibers. Collagen and elastic fibers are more prominent. Small amount of fatty tissue is seen just beneath the epicardium. Every section of SA node in infant and adolescent is spindle-shaped or arrowhead-like. Panel C: SA node of an 81-year-old elderly patient (case 24): SA nodal cells are atrophic and sparse, fibrous connective tissues are more distinct, and the peripheral region of the SA node is irregularly infiltrated with fatty tissue. These changes are most remarkable in the lateral side adjacent to the epicardium. The shape of SA node is irregular and polygonal. (Objective lens: SPlan-Apo ×4; bars, 500 μm).

Figure 3. Graph shows volumes of the total sinoatrial (SA) node, SA nodal cells, and fibrous connective tissue. Until young adulthood, all components of the SA node increase and the total SA node size also increases. The connective tissue and other connective components increase to a greater degree than do the SA nodal cells. The volume of total SA node and SA nodal cells decreases in the elderly.
Volume Changes During Growth

The volume of the SA nodal cells significantly increased until adulthood. The mean volume in adolescents and adults was 2.4-fold greater than that in infants (Figure 5A). The volume ratio of SA nodal cells to the total SA node, however, became less during growth; the ratios were 39.0±7.3% in infants and 21.5±2.9% in adolescents and adults (Figure 5B). The reason is apparently the 7.4-fold increase in volume of the connective tissue during growth. The phenomenon has been mixed up to an actual loss of SA nodal cells. The fibrous infiltration observed during growth is presumably a normal process in growing hearts, probably resulting in structural or electrophysiological stabilization. A recent study concerning the human atrioventricular (AV) nodal cell reported the same conclusion (unpublished data).

The SA nodal cells are a more uniform, small population. Although the number of polyploid working myocardial cells increased with age,18 neither octaploid nuclei nor any other change of DNA ploidy pattern during growth was present in the SA nodal cells. This result indicates that the SA nodal cells neither synthesize DNA nor proliferate after birth, as is also the case with the AV nodal cells.20 These results could not be obtained. The youngest subject in this study was a 3-month-old infant. In most of the elderly SA nodes, the peripheral areas and surrounding right atrial myocardium were infiltrated with fatty tissue (Figure 2C). The area of elderly SA node was defined to the remaining tissue excluding the surrounding fatty tissue.
suggest that the pattern of physiological growth of the SA nodal cells is simple hypertrophy, whereas that of working myocardial cells is hypertrophy with nuclear polypliodization.

**Volume Changes in the Elderly**

The volume of SA nodal cells in the elderly over 50 years of age significantly decreased compared with that in adolescents and adults (Figure 5A). The volume percentage of SA nodal cells in the total SA node also decreased (Figure 5B, 16.6±2.3%). Reduction in the size of the SA node and/or shrinkage in the size of individual SA nodal cells might be responsible for the volume loss of the nodal cells in the elderly cases. Serial sections of the elderly SA node (Figure 2C) demonstrated irregular loss of the peripheral areas of the SA node and resultant replacement of fatty tissue. Actually, the calculated total volume of SA node significantly decreased during aging (Figure 4). The equations for reduction ratios of the volume given as (volume in adolescents and adults−volume in the elderly)/volume in adolescents and adults were 21.4% in the total SA node and 38.9% in SA nodal cells. The discrepancy probably results from the shrinkage in the size of individual SA nodal cells. The reduction in the volume percentage of SA nodal cells in the elderly (Figure 5B) also implies atrophy of each SA nodal cell.

The calculated total volume of fibrous connective tissue by no means increased during aging (Figure 6A), although serial sections of the elderly SA node (Figure 2C) revealed remarkable fibrous connective tissue, and the volume percentage of fibrous connective tissue increased (Figure 6B, 49.3±5.8%). This reduction in size of the SA node is caused by loss of its peripheral area, which could reduce the original volume of the connective tissue, although the fibrous connective tissue might replace atrophy of individual SA nodal cells, i.e., fibrosis.

Atrophy of SA nodal cells, loss of the SA node, and/or infiltration of fatty tissue may have a relation to SA nodal dysfunction that increases with age in the elderly. The quantitative volume analysis of the SA node and its components with sinus node dysfunction should be a subject of further research.

**Table 2. DNA Contents of Sinoatrial Nodal Cells and Right Atrial Myocardial Cells**

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>RA myocardial cells (%)</th>
<th>SA nodal cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2C 4C 8C</td>
<td>2C 4C 8C</td>
</tr>
<tr>
<td>1</td>
<td>3 Months</td>
<td>93  7  0</td>
<td>95  5  0</td>
</tr>
<tr>
<td>2</td>
<td>7 Years</td>
<td>89  10  2</td>
<td>97  3  0</td>
</tr>
<tr>
<td>3</td>
<td>39 Years</td>
<td>90  8  2</td>
<td>95  5  0</td>
</tr>
</tbody>
</table>

RA, right atrial; SA, sinoatrial.
References

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